Structural Features in the KshA Terminal Oxygenase Protein That Determine Substrate Preference of 3-Ketosteroid 9 alpha-Hydroxylase Enzymes

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Supplemental Table 2. Primers used in this study

Chimer	Template	Primer name	Sequence (5'-3')
	kshA1	KshA1-c768g-F[a]	GGATACGACGTCGAGTCGGTCCTGATCAACTGCCACTACC
		KshA1-c768g-R[a]	GGTAGTGGCAGTTGATCAGGACCGACTCGACGTCGTATCC
	kshA5	KshA5-g612a-F[a]	AAGAACGTCTTCGAAGGCCACACCGCCACGCAG
		KshA5-g612a-R[a]	TGCGTGGCGGTGTGGCCTTCGAAGACGTTCTTG
	kshA5	KshA5-c786g-F[a]	CGAGACCATCCTGATCAACTGCCACTACCCGGTGAGC
		KshA5-c786g-R[a]	GCTCACCGGGTAGTGGCAGTTGATCAGGATGGTCTCG
kshA1$_{A5loop}$, *kshA1*$_{A5TG}$	*kshA1*	pA1A5-13-F2	CGATCCGATGCAAGCTATTT
		pA1A5-13-R2 long	TCCTCACGACCGGTACCGCGCATGAACTGGCTGG
	kshA1	pA1A5-13-F2 long	AACGCCGAACTGCGATCCGATGCAAGCTATTT
		pA1A5-13-R2	ACCGCGCATGAACTGGCTGG
	kshA5	pA1A5-13-F1	ACCGGTCGTGAGGACGTCAT
		pA1A5-13-R1 long	TTGCATCGGATCGCAGTTCGGCGTTGGGGTCGTC
	kshA5	pA1A5-13-F1 long	AGTTCATGCGCGGTACCGGTCGTGAGGACGTCAT
		pA1A5-13-R1	CAGTTCGGCGTTGGGGTCGTC
kshA5$_{A1loop}$	*kshA5*	pA1A5-16-F2	CGTTCCGAGGCAACCTATTT
		pA1A5-16R2 long	CTCACGGGCCTGCGAGTGCATGTACTGCGTGG
	kshA5	pA1A5-16-F2 long	ATGATCGGAAGCCGTTCCGAGGCAACCTATTT
		pA1A5-16-R2	CGAGTGCATGTACTGCGTGG
	kshA1	pA1A5-16-F1	CAGGCCCGTGAGGACACCCGTCCG
		pA1A5-16-R1 long	TGCCTCGGAACGGCTTCCGATCATCTTCGGTTGA
	kshA1	pA1A5-16-F1 long	TACATGCACTCGCAGGCCCGTGAGGACACCC
		pA1A5-16-R1	GCTTCCGATCATCTTCGGTTGA
kshA1$_{A5\beta201-210}$	*kshA1*	pA1A5-19-F[b]	phos-**ACCGCCACGCAGTACATGCACTCG**CAGGCCCGTGAGGACA
		pA1A5-19-R	GTGTCCTTCGAAGACGTTCTTGAAGTACACCGGGAAGGAG
kshA5$_{A1\beta207-216}$	*kshA5*	pA1A5-22-F[b]	phos-**GTCGCCAGCCAGTTCATGCGCGGT**ACCGGTCGTGAGGAC
		pA1A5-22-R	GTGGCCCTCGAAGACGTTCTTGAAGTAGCGCGGGAACGA
kshA1$_{A5\beta230-238}$	*kshA1*	pA1A5-25-F[b]	phos-**GGCAACCTATTTCGGTCCGTCGTA**CATGATCGACGATCTCG
		pA1A5-25-R	TCGGATCGGCTTCCGATCATCTTCGGTTGACCGTTCG
kshA5$_{A1\beta236-244}$	*kshA5*	pA1A5-28-F[b]	phos-**TGCAAGCTATTTCGGCCCGTCCTT**CATGATCGACTGGCTC
		pA1A5-28-R	TCGGAACGCAGTTCGGCGTTGGGGTCGTCGTAGTTGGT
kshA1$_{D242W}$	*kshA1*	KshA1-D242W-F[a]	CCCGTCCTTCATGATCGACTGGCTCGTCTACGAGTACGAG
		KshA1-D242W-R[a]	CTCGTACTCGTAGACGAGCCAGTCGATCATGAAGGACGGG
kshA5$_{W248D}$	*kshA5*	KshA5-W248D-F[a]	TCCGTCGTACATGATCGACGATCTCGAATCCGATGCCAAC
		KshA5-W248D-R[a]	GTTGGCATCGGATTCGAGATCGTCGATCATGTACGACGGA

[a]Mutated residues are underlined, [b]the stretch of residues corresponding to *kshA5* (pA1A5-19-F and pA1A5-25-F) or *kshA1* (pA1A5-22-F or pA1A5-28-F) is shown in bold.